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Risk factors for coronary artery disease, circulating endothelial progenitor cells, and the role of HMG-CoA reductase inhibitors

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Risk factors for coronary artery disease, circulating endothelial progenitor cells, and the role of HMG-CoA reductase inhibitors. Recent studies suggest that postnatal neovascularization relies not exclusively on sprouting of preexisting vessels ("angiogenesis"), but also involves the contribution of bone marrow–derived circulating endothelial progenitor cells (EPCs). EPCs can be isolated from peripheral blood or bone marrow mononuclear cells, $CD34^+$ or $CD133^+$ hematopoietic progenitors. Infusion of EPCs was shown to promote postnatal neovascularization of ischemic tissue after myocardial infarction in animal models and initial clinical trials. Moreover, circulating endothelial precursor cells can home to denuded arteries after balloon injury and contribute to endothelial regeneration, thereby limiting the development of restenosis. Thus, circulating endothelial cells may exert an important function as endogenous repair mechanism to maintain the integrity of the endothelial monolayer and to promote ischemia-induced neovascularization. However, risk factors for coronary artery disease, such as diabetes, hypercholesterolemia, and hypertension are associated with impaired number and function of EPC in patients with coronary artery disease. Therapeutically, the reduction of EPC number and the decreased functional activity in patients with coronary artery disease was counteracted by 3-hydroxy-3-methylglutaryl coenzymeA (HMG-CoA) reductase inhibitors (statins), vascular endothelial growth factor (VEGF), estrogen, or exercise. At the molecular level, these factors are well established to activate the phosphatidyl-inositol-3-kinase (PI3K)-Akt-dependent activation of the endothelial nitric oxide synthase (eNOS), suggesting that the PI3K-Akt-eNOS signaling pathway may be involved in the transduction of atheroprotective factors. Taken together, the balance of atheroprotective and proatherosclerotic factors may influence EPC levels and their functional capacity to improve neovascularization and endothelial regeneration.

CHARACTERIZATION AND FUNCTION OF ENDOTHELIAL PROGENITOR CELLS (EPCs)

The growth of blood vessels to provide oxygen supply for ischemic tissues or tumors in the adult is mediated by arteriogenesis, angiogenesis or vasculogenesis [1]. Arteriogenesis refers to the formation of collateral vessels, whereas angiogenesis is defined as the growth of new capillaries by sprouting of preexisting vessels through migration and proliferation of mature endothelial cells. The concept of vasculogenesis was originally described as de novo blood vessel formation in embryonic development. In this context, endothelial precursor cells were defined as "angioblasts." Meanwhile, vasculogenesis also refers to adult blood vessel formation involving the mobilization of bone marrow–derived endothelial progenitor cells, which home to sites of ischemia and contribute to new blood vessel formation [1]. The finding that vasculogenesis contributes to blood vessel formation in the adult offers novel therapeutic strategies for the use of circulating EPCs or their precursors for cell therapy of tissue ischemia [2–5]. Initial studies by Asahara et al [6] and Shi et al [7] demonstrated that bone marrow–derived hematopoietic progenitor cells can give rise to endothelial cells and contribute to endothelial recovery after balloon injury and new capillary formation after ischemia. In addition, EPCs can be grown from peripheral blood mononuclear cells (MNCs) or purified populations of CD34-positive or CD133-positive hematopoietic cells [6, 8]. Furthermore, CD14-positive MNCs have been used as starting population for cultivation of EPCs [9]. EPCs were characterized by the coexpression of the hematopoietic marker CD34 and endothelial marker proteins such as vascular endothelial growth factor receptor-2 (VEGFR-2), von Willebrand factor, VE-cadherin, CD146, and CD31 [4, 6, 10]. Moreover, these cells were defined by their functional capacity to form endothelial cell colonies and enhanced endothelial nitric oxide synthase (eNOS) expression after shear stress exposure [7, 11, 12].

The finding that bone marrow–derived cells can home to sites of ischemia and express endothelial marker proteins has initiated a variety of experimental studies investigating the function of EPCs in vivo. Infusion of ex vivo expanded peripheral blood-derived EPCs in nude mice or rats improved the neovascularization in hind limb ischemia models [4, 13–15]. Moreover, in animal models of myocardial infarction, the injection of ex vivo expanded EPCs or stem- and progenitor cells significantly improved blood flow and reduced left ventricular scarring [14, 16]. Similarly, initial pilot trials indicate that bone

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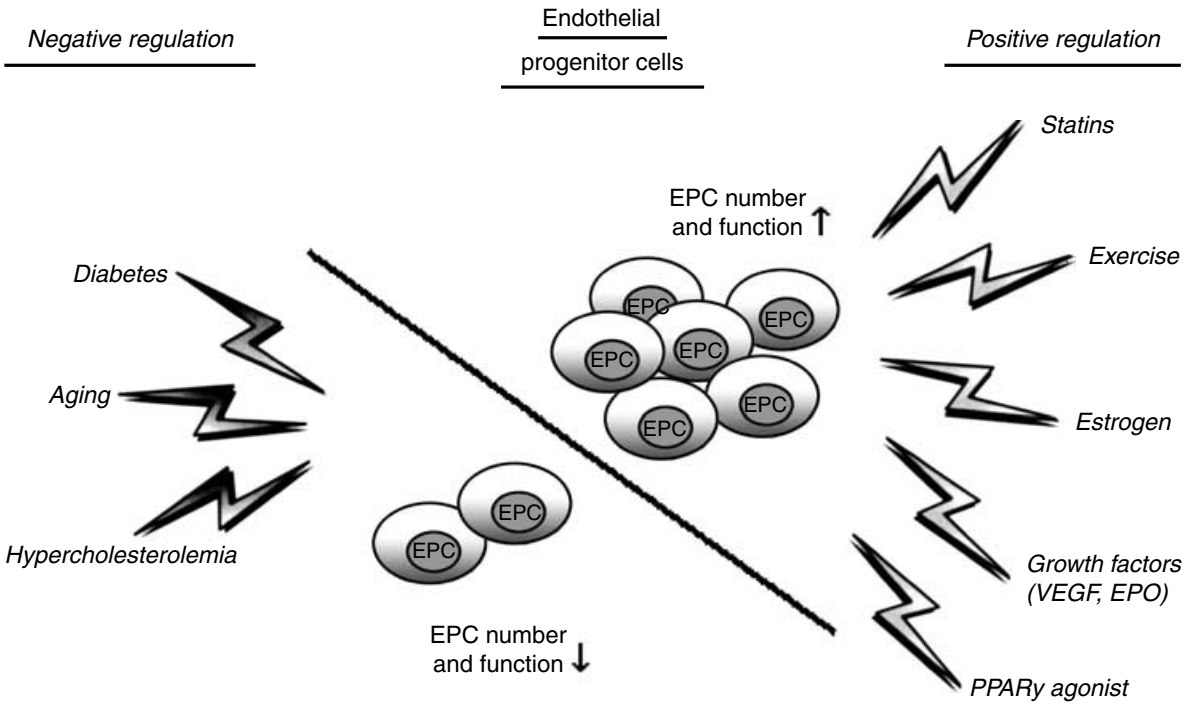

Fig. 1. Effect of risk factors for coronary artery disease on endothelial progenitor cell (EPC) number and function and potential counteraction. Abbreviations are: VEGF, vascular endothelial growth factor; EPO, erythropoietin; PPAR-γ, peroxisome proliferators-activated receptor-γ.

marrow–derived or circulating blood–derived progenitor cells are useful for therapeutically improving blood supply of ischemic tissue [5, 17].

The maintenance of the integrity and the functional activity of the endothelial monolayer are of crucial importance to prevent atherogenesis. Increasing evidence suggests that risk factors for coronary artery disease enhance endothelial cell apoptosis, thereby, disturbing the endothelial monolayer [18–20]. In the past, the regeneration of injured endothelium has been attributed to the migration and proliferation of mature endothelial cells. However, recent studies indicate that the injured endothelial monolayer may also be regenerated by circulating endothelial cells. Indeed, in 1998 Shi et al [7] have shown that bone marrow–derived cells deriving from $CD34^+$ hematopoietic stem cells can colonize endothelial flow surfaces of vascular prostheses in a dog model. In humans, the surface of ventricular assist devices was covered by $CD133^+/VEGFR2^+$ hematopoietic stem cells [21]. EPCs as well as bone marrow–derived cells home to denuded arteries after balloon injury, accelerate reendothelialization and reduced neointima formation and restenosis (for review see [22]). Interestingly, a recent study demonstrated that apoptotic bodies from mature endothelial cells augmented the number and differentiation of endothelial progenitor cells postulating an endogenous mechanism to facilitate the repair of injured endothelium [23]. Whereas the regeneration of the endothelium by EPC protects atherosclerotic lesion formation, bone marrow–derived stem or progenitor cells may also contribute to plaque angiogenesis, thereby potentially promoting plaque instability [24]. However, in a recent study, no influence of bone marrow cell infusion on plaque composition was detected in nonischemic mice [25]. An increased plaque size was only detected in the response to ischemia suggesting that ischemia-induced release of growth factors contribute to this effect [25].

RISK FACTORS AND EPC

In patients with coronary artery disease the number of circulating EPCs is significantly reduced [12] (Fig. 1). Classic risk factors for atherosclerosis such as age or smoking inversely correlate with the number of circulating CD34/KDR- or CD133/KDR-positive cells [12]. Likewise, the number of ex vivo cultivated peripheral blood–derived EPCs and the migratory response of this cultivated EPCs toward VEGF was significantly reduced in patients with coronary artery disease [12]. Diabetes, which is a predominant risk factor for coronary artery disease, impairs $CD34^+$ hematopoietic precursor cells as well as EPC number and function [26–28]. Thus, two recent studies have shown that patients with type 1

[27] or type 2 diabetes [28] revealed lower numbers of EPCs as assessed by outgrowth assays. Both studies have shown that the number of EPCs inversely correlated with haemoglobin A_{1c} (HbA_{1c}) in type 1 and type 2 diabetes [27, 28]. Moreover, EPCs of type 2 diabetic patients are impaired in adhesion to the endothelium, proliferation, and tubulization [28]. Interestingly, the dysfunction of type 1 diabetic patient-derived EPCs was not reversed by cultivation in normoglycemic medium. These data suggest that the impairment of EPCs is not reversible by removal of one harmful factor [27]. The inflammatory marker protein C-reactive protein (CRP), which is elevated in patients with coronary artery disease and diabetes, also impaired EPC function and induced EPC apoptosis in vitro [29].

Interestingly, in healthy subjects classic risk factors for atherogenesis are also associated with a reduction of peripheral blood–derived endothelial cells [30]. Moreover, the number of outgrowing endothelial colonies correlated with the endothelial function as assessed by measurement of flow-dependent dilation, which is a prognostic parameter for the functional activity of the endothelium in patients with coronary artery disease [30]. First evidence that the reduction of EPCs may have an impact on neovascularization was provided by a recent study of Lambiase et al [31]. Thereby, an inadequate coronary collateral development was associated with reduced numbers of EPCs and impaired chemotactic and proangiogenic activity suggesting that EPCs may contribute to functional collateral formation [31]. Taken together, the reduction of EPCs by risk factors may contribute to a vicious cycle resulting in reduced endothelial regeneration and function, and impaired neovascularization.

EFFECT OF 3-HYDROXY-3-METHYLGLUTARYL COENZYME A (HMG-COA) REDUCTASE INHIBITORS (STATINS)

A variety of factors, including cytokines [e.g., VEGF, erythropoietin (EPO), and granulocyte macrophage-colony-stimulating factor (GM-CSF)], hormones such as estrogen, and pharmacologic substances like statins and peroxisome proliferators-activated receptor-γ (PPAR-γ) agonists as well as physical training regulate EPC numbers [3, 10, 29, 32–35] (Fig. 1). Particularly, HMG-CoA reductase inhibitors increased the number and the functional activity of EPCs in vitro [10, 36], in mice [10, 36], and in patients with stable coronary artery disease [37]. Moreover, statin therapy accelerates reendothelialization after balloon injury by improving mobilization and incorporation of bone marrow–derived EPCs [38, 39]. The lipid-lowering effect of statins may contribute to the regulation of EPC number and function. However, at least in mice, the beneficial effects of statins were observed without a change in serum cholesterol levels [40]. Beside lipid lowering statins exert a variety of pleiotropic effects, including the increase of expression and activity of eNOS (for review see [41]). These pleiotropic effects of statins might influence mobilization, proliferation and apoptosis of EPCs [10, 36, 42]. Particularly, eNOS appears to contribute to mobilization and functional activity of EPCs [43]. Thus, the increase in EPC mobilization and cardiac function by statins was abolished in eNOS-deficient mice [40]. However, statins additionally affected the aging of EPC and delayed the onset of senescence at least in part independently on nitric oxide [42, 44]. The increased expression of the telomere repeat binding factor-2 induced by statins during ex vivo cultivation of EPCs may contribute to the delayed senescence and functional improvement of EPCs [44].

At the molecular level, several studies indicate that the prosurvival phosphatidyl-inositol-3-kinase (PI3K)/Akt pathway may play an important role not only in mature endothelial cells [45] but also in EPCs [10, 42]. Thus, statins, VEGF, EPO, estrogen, and exercise (shear stress) are well known to augment the PI3K/Akt-pathway [46–50]. Based on the finding that eNOS is essential for mobilization of bone marrow–derived stem and progenitor cells [43], one may speculate that these stimuli may increase progenitor cell mobilization by PI3K/Akt-dependent activation of the NOS within the bone marrow stromal cells. Consistent with the requirement of eNOS for statin-induced mobilization, exercise- and VEGF-timulated EPC mobilization was also abolished in eNOS-deficient mice [35, 40, 43].

CONCLUSION

EPCs significantly contribute to adult neovascularization after ischemia and to endothelial repair after injury. Risk factors for coronary artery disease impair EPC number and function. For therapeutic cell therapy the reduction of EPC number was counteracted by the pharmacologic intervention with statins, cytokines (VEGF and EPO), estrogen, and physical activity (exercise). All these factors activate the PI3K/Akt pathway. Further downstream the eNOS seems to play an important role since statin-, exercise-, and VEGF-induced mobilization of EPCs is blunted in $eNOS^{-/-}$ mice. Understanding the molecular mechanism will help to modulate EPC number and function in order to optimize cell therapy for a variety of vascular diseases, including coronary artery and renal diseases.

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